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The urinary excretion of γ-hydroxybutyric acid in man

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Abstract

γ-Hydroxybutyric acid (GHB) has been widely associated with drug-facilitated sexual assault (DFSA). However, its excretion profile in man has not been well characterized. To assess the detectability of GHB for forensic cases and to correlate urinary levels with dose, we have examined the excretion profiles of 1- and 2-g doses of GHB (sodium salt) in a healthy male volunteer. The urinary levels were measured by a novel, simple and highly reproducible method. The drug was found to be excreted in small amounts in the free form (0.86 and 1.16 % for 1- and 2-g doses, respectively) rapidly in urine ($\leqslant$ 10 h). The urinary levels were found to be in the low mg L^{-1} range (up to 29.1 mg L^{-1}). The work presented demonstrates that it is of the utmost importance to collect the samples as soon as possible following the alleged assault.

Introduction

γ-Hydroxybutyric acid (GHB) has been identified in drug-facilitated sexual assault (DFSA). Although alcohol is the most widely implicated drug in such cases, GHB has been found in a sizeable proportion of samples (3 %) (Slaughter 2000). This is remarkably high as the drug is essentially illicit whereas alcohol is not normally used as a primary DFSA agent but simply forms part of a scenario where the victim has had too much to drink (possibly by his/her own choice) and hence becomes more vulnerable. The fact that GHB is found in a urine sample would indicate that its intended use was purely for DFSA.

GHB is water soluble, producing solutions that are mildly saline (and easily disguised) and is quick acting. For our routine forensic testing program we required a method for its detection/quantification in urine.

GHB is a scheduled drug (S1A) in Ireland, which means that a prescription from a clinician is required and it can only be dispensed once, i.e. a repeat needs another prescription (The Irish Pharmacy Journal 1999). No licensed medical preparation is currently available. However, it is readily available as a laboratory chemical and is relatively cheap (33 Euros for 25 g in June 2000). Its preparation from γ-butyrolactone is well documented on the internet (www.erowid.org/chemicals/ghb/ghb_synthesis.shtml). The only medical application for GHB has involved its use in research into the treatment of alcohol dependence using doses varying from 25 to 50 mg kg^{-1} (Ferrara et al 1992). At 50 mg kg^{-1} and taking an average body mass of 70 kg, this would result in an overall dose of 3.5 g, although it is difficult to identify doses used in DFSA due to the varied sources and uncontrolled nature of the products available.

GHB is eliminated rapidly from the body and it has been reported that only negligible amounts (approximately 1 %) are excreted unchanged in the urine

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(Ferrara et al 1992, 1993). However, the actual urinary levels are not discussed. It has been shown that at a dose of 25 mg kg^{-1} the drug is undetectable in urine after 8 h, a worrying fact considering that most people would provide a urine sample for testing the day after the incident (> 8 h) (Ferrara et al 1992, 1993). However, McCusker et al (1999) reported that large concentrations had been found in the urine of DFSA victims. In one case the level was found to be 6.1 g L^{-1} (range 2.3 mg–6.1 g L^{-1}). Although a single sample is only a snapshot of the whole excretion profile picture, as a rough estimate, if one takes the rather modest volume of urine voided as 40 mL to obtain this sample (in the work presented here we found that voidings ranged from 40 to 635 mL), then this would contain 244 mg GHB. If only 1 % is excreted unchanged and all of this was in the single voiding (a gross underestimate) then the ingested dose would be in the order of 24.4 g. It has been reported that 5–10 g can be toxic or even fatal so there may be a narrow margin between any pharmacological effect and toxicity (www.erowid.org/chemicals/ghb/ghb_dose.shtml).

GHB has a half-life in the order of 30 min. In the present study, blood was not collected because the intention of the work was to establish the longest time at which GHB could be detected in a bio-fluid, thus making urine a better choice. In our laboratory, urine is frequently the only bio-fluid presented for analysis. For example, we recommend self-collection as soon as possible after the incident so urine would be the only readily available bio-fluid.

The literature appears to be somewhat confusing and this prompted us to examine the excretion of GHB in man to relate its detectability to forensic cases. Although dose has been correlated with effect, there are no reports of how much has actually been used in DFSA (www.erowid.org/chemicals/ghb/ghb_dose.shtml). It is also unclear as to what the typical urinary levels would be following a given dose.

Materials and Methods

Administration

γ-Hydroxybutyric acid, sodium salt (1- and 2-g doses administered one week apart), dissolved in water (30 mL), was given orally to a healthy male volunteer (approximately 100 kg, 1-g dose equivalent to 10 mg kg^{-1}, 2 g equivalent to 20 mg kg^{-1}). The volunteer consumed approximately 1.5 L water in the 30 min immediately before the administration. A pre-administration urine sample was collected. Urine samples were collected at intervals afterwards. All samples were stored at -20°C until analysis.

Reagents

The derivatizing agent was prepared by mixing together *tert*-butyldimethylsilyl chloride (50 mg), acetonitrile (500 μL) and *tert*-butyldimethylsilyltrifluoroacetamide (500 μL). The pH 2 buffer consisted of 0.05 M potassium chloride adjusted to pH 2 with HCl. Bromocresol green solution (1 %) was prepared in methanol.

Internal standard

The internal standard was prepared by heating γ-caprolactone (100 μL, Aldrich cat. no. 30,383-6) and 2 M aqueous hydroxide (2 mL) at 80–90°C for 30 min, diluting to 90–95 mL with distilled water, adjusting to pH 7–7.5 and making up to 100 mL with distilled water.

Extraction

A mixture of urine (1 mL), bromocresol green solution (20 μL; after diluting with pH 2 buffer, the final solution should be yellow. If it remains blue, 0.05 M HCl is added until the mixture is yellow), internal standard solution (20 μL) and pH 2 buffer (9 mL) was extracted with TBME (*tert*-butylmethylether: 4 mL) by rotating for 20 min. The mixture was then centrifuged (3000 rev min^{-1}), the upper layer collected and blown to dryness at 40°C with nitrogen. The residue was reconstituted with 75 μL derivatizing agent. Samples were analysed in duplicate.

GC/MS

GC/MS was performed on an Agilent 5973 MSD (EI, 70 eV, TIC mode with m/e 100–370, m/e 275 monitored for GHB and m/e 303 for the internal standard, both M-*t*-Bu) coupled to a 6890 GC. Samples (1 μL) were injected in splitless mode with the following conditions: 50°C for 1 min, 10°C min^{-1} to 300°C using a polydimethylsiloxane column (HP1 or equivalent, 25 m × 0.32 mm, 0.5 μm coating; the choice of column is not critical. Most polydimethylsiloxane and 5 % phenyl/95 % dimethylsiloxane copolymer columns will work) and helium at 1 mL min^{-1}.

The linear working range ($r^2 > 0.999$ using peak ratios) was found to be 2–100 mg L^{-1} with intraday and interday CV values of 4.1 and 6.9 % for a 2 mg L^{-1} standard. The detection threshold for practical purposes

was set at 2 mg L^{-1} although it would be possible to reach a much lower value.

Results and Discussion

At the outset of the work it was felt important to set a detection threshold for the presence of the drug. It has been reported that GHB is not detectable in normal urine (Fiel et al 1998). However, our approach was to set a value that would be indicative of the drug's administration and not the ultimate limit of detection. The reporting limit is a threshold to identify the administration of the drug as opposed to any potential background level/interference. A commercial laboratory (National Medical Services, Willow Grove, PA, www.nmslabs.com) that provides a testing service for GHB quotes a reporting limit of 2 μg mL^{-1} (indicating negative/positive), which we adopted also.

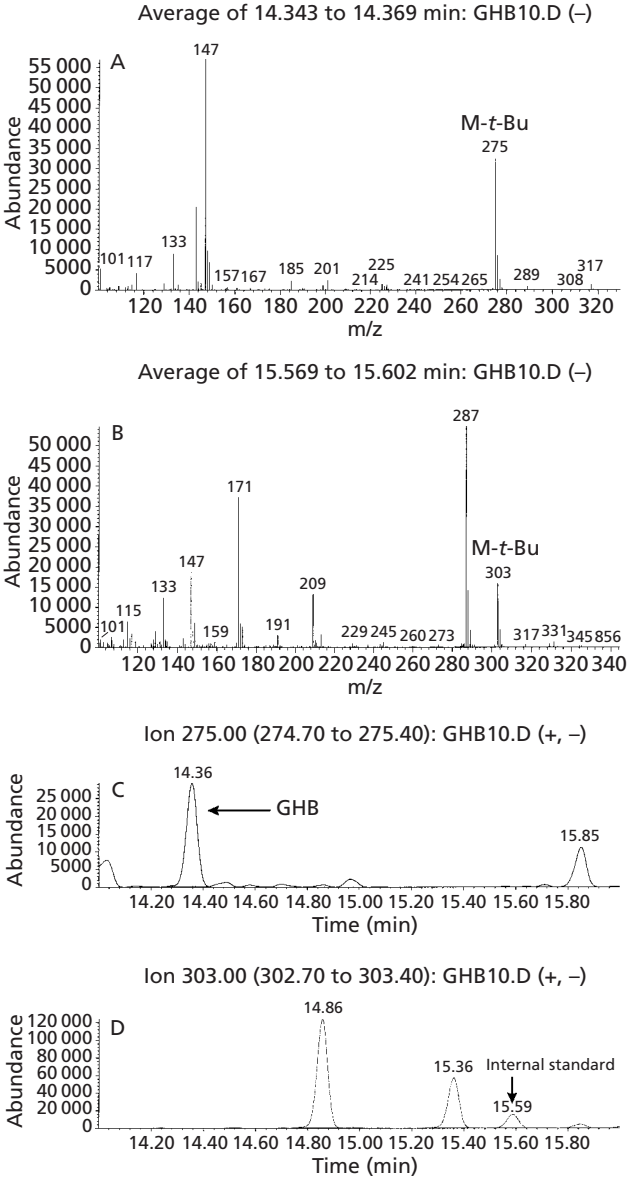

Figure 1 A. EI mass spectrum of GHB (TBDMS (*tert*-butyldimethylsilyl) derivative). B. EI mass spectrum of internal standard (TBDMS derivative). C. EIC (m/e 275) for GHB (TBDMS derivative) at 14.36 min and D. EIC (m/e 303) for GHB (TBDMS derivative) at 15.59 min. M-*t*-Bu, *tert*-butyldimethylsilyl group.

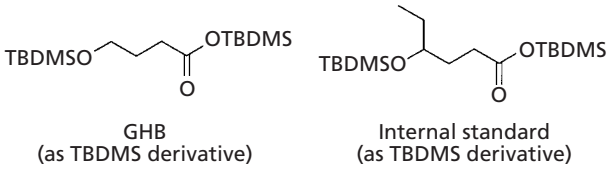

Figure 2 The structures of GHB and the internal standard.

Having evaluated methods using ethyl acetate extraction and monitoring the M-Me (m/e 233) ion following TMS (trimethyl-silyl) derivatization (Elian 2000) we found that, although the mass spectral quality was adequate, there was interference in some samples from a peak (not characterized) eluting just before the GHB. This was much more intense and had a base peak of m/e 231, with its m/e 233 isotope peak obscuring the GHB. Methyl *tert*-butyl ether (MTBE) was then evaluated as a solvent and found to give much cleaner extracts. Sample preparation was performed by simply adding a pH 2 buffer to the urine and using a pH indicator to show that the sample was sufficiently acidic.

We investigated the use of the *tert*-butyldimethylsilyl group, which proved successful giving a suitable ion of mass 275 (M-*t*-Bu) (Figure 1). The higher mass also allowed better discrimination from the background. Its intensity was comparable with m/e 233 for the TMS derivative. The ideal internal standard would have been d_6-GHB but this was too cost prohibitive (55 Euros for 100 μg). The ethyl analogue of GHB (Figure 2) was used instead and gave excellent reproducibility ($r^2 > 0.999$).

The doses of GHB (1 and 2 g, as sodium salt) administered were kept low but in the magnitude of typical amounts reported as being 'common' (www.erowid.org/chemicals/ghb_ghb-dose.shtml). The excretion profiles are shown in Table 1. Just before the administration, a large quantity of water was consumed to simulate the intake of fluid that would be encountered by potential victims in a social setting (e.g. a bar or a club). Large quantities of urine voided soon after the drug had been ingested would be expected in such a situation and this was indeed found. Although

Table 1 Excretion profiles for doses of GHB (1 and 2 g).

1 g GHB		2 g GHB	
Time after dosing (h)	Concn (mg L^{-1})	Time after dosing (h)	Concn (mg L^{-1})
0	ND*	0	ND
1	7.7	1	14.0
1.5	16.5	2	29.1
10	4.0	10	< 2 (1.9)
16	< 2 (1.7)	18, 22, 25, 33	ND
21, 25, 36	ND		
% excreted (0–10 h): 0.86%**		% excreted (0–2 h): 1.16%**	

*The actual limit of detection for the assay was estimated to be approximately 0.2 mg L^{-1}. **For concentrations > 2 μg mL^{-1}.

the doses did not induce sleep, there was an euphoric effect making the volunteer feel relaxed and open to increased social interaction. Such a situation could make a potential victim quite vulnerable.

The drug was found to be excreted very quickly (Table 1). In the case of the 1-g dose it was detectable for up to 10 h (using the 2 μg mL^{-1} cut-off) and only up to 2 h following the 2-g dose. As reported previously (Ferrara et al 1992, 1993), only approximately 1% (0.86% and 1.16% for the 1- and 2-g doses, respectively) was found to be excreted unchanged, suggesting that other metabolites/conjugates should be targeted to extend the detection time.

In conclusion, it was observed that it is essential to collect the urine specimen as soon as possible after the incident. The method presented here was found to be robust, reliable and reproducible. Even the morning following the DFSA incident, it could be too late to reliably identify the presence of the drug. A major determining factor on the urinary concentration is the volume voided. As GHB is likely to be encountered at social gatherings where large quantities of beverages are consumed, this may make its detection more complicated due to urinary dilution.

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